Effects of Dietary Neutral Detergent Fiber Level on Rumen Bacterial Structure and Composition in Goats (Postprint)

Authors: Zhang Xuejiao, Wang Lizhi

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Abstract

This study aimed to investigate the effects of dietary neutral detergent fiber (NDF) levels on rumen bacterial structure and composition in goats using high-throughput sequencing technology. Six goats were selected for a 3×3 Latin square experiment and divided into low (35.01%, LN group), medium (40.10%, MN group), and high NDF level groups (45.16%, HN group) based on dietary NDF levels, with 2 goats per group. The feeding trial was conducted in 3 periods, with each period lasting 20 d, including a 14-d preliminary period and a 6-d formal experimental period. After the formal experimental period, rumen contents were collected from the goats, bacterial total DNA was extracted, the V4 region of 16S rRNA was amplified by PCR using bacterial universal primers, the amplicons were subjected to high-throughput sequencing on the Illumina HiSeq 250PE platform, and the sequencing results were analyzed using bioinformatics software such as QIIME 1.8.0. The results showed that: 1) The rumen fluid ammonia nitrogen (NH3-N) concentration in the HN group was extremely significantly lower than that in the LN and MN groups ($P<0.01$); the acetate/propionate ratio in rumen fluid of the LN group was significantly lower than that of the HN group ($P<0.05$), but there was no significant difference between the MN group and the other two groups ($P>0.05$). 2) There were no significant differences in Chao1 and Shannon indices among the groups ($P>0.05$); the observed species index in the LN group was significantly higher than that in the other two groups ($P<0.05$), while there was no significant difference between the other two groups ($P>0.05$). 3) At the phylum level, there were no significant differences in the relative abundance of all bacteria among the three groups ($P>0.05$); at the genus level, the relative abundances of Prevotellaceae UCG-001, Prevotellaceae UCG-003, and Ruminococcaceae UCG-014 in the HN group were significantly higher than those in the other two groups ($P<0.05$); the relative abundances of Ruminococcaceae NK4A214 group and Ruminococcaceae UCG-005 in the HN group were significantly higher than those in

the LN group (P<0.05); the relative abundances of SP3-e08 and Lachnoclostridium 10 in the HN group were significantly lower than those in the other two groups (P<0.05); the relative abundance of Succiniclasticum in the LN group was significantly higher than that in the other two groups (P<0.05); the relative abundances of Lysinibacillus, Bacillus, and Phyllobacterium in the MN group were significantly higher than those in the other two groups (P<0.05), and the relative abundance of Victivallis was extremely significantly higher than that in the other two groups (P<0.01). In conclusion, when dietary NDF levels varied from 35.01% to 45.16%, they significantly affected rumen fluid NH3-N concentration and acetate/propionate ratio, and significantly influenced the relative abundances of various bacterial genera such as Prevotellaceae UCG-001 and Prevotellaceae UCG-003 in the rumen.

Full Text

Effects of Dietary Neutral Detergent Fibre Level on Structure and Composition of Rumen Bacteria in Goats

ZHANG Xuejiao, WANG Lizhi*

Animal Nutrition Institute, Sichuan Agricultural University, Chengdu 611130, China

Abstract: This study investigated the effects of dietary neutral detergent fibre (NDF) level on the structure and composition of rumen bacteria in goats using high-throughput sequencing technology. Six healthy male Nubian goats were allocated to a 3 × 3 Latin square design with three dietary treatments based on NDF level: low (LN group, 35.01%), medium (MN group, 40.10%), and high (HN group, 45.16%), with two goats per group. The feeding trial consisted of three periods, each lasting 20 days (14-day pre-trial and 6-day formal trial). Following each formal trial period, rumen contents were collected for bacterial total DNA extraction. The V4 region of the bacterial 16S rRNA gene was amplified using universal primers and sequenced on the Illumina HiSeq 250PE platform. Bioinformatics analysis was performed using QIIME 1.8.0 and other software. The results showed: (1) Rumen ammonia nitrogen (NH -N) concentration in the HN group was significantly lower than in the LN and MN groups ($P < 0.01$). The acetate-to-propionate ratio in the LN group was significantly lower than in the HN group ($P < 0.05$), while the MN group showed no significant difference from the other two groups ($P > 0.05$). (2) No significant differences were observed among groups for the Chao1 and Shannon indices ($P > 0.05$). However, the observed species index in the LN group was significantly higher than in the other two groups ($P < 0.05$), with no significant difference between the MN and HN groups ($P > 0.05$). (3) At the phylum level, no significant differences were detected in bacterial relative abundance among the three groups ($P > 0.05$). At the genus level, the relative abundances of *Prevotellaceae* UCG-001, *Prevotellaceae* UCG-003, and *Ruminococcaceae* UCG-014 in the HN group were significantly higher than in the other two groups ($P < 0.05$). The relative

abundances of *Ruminococcaceae* NK4A214 group and *Ruminococcaceae* UCG-005 in the HN group were significantly higher than in the LN group ($P < 0.05$). Conversely, the relative abundances of SP3-e08 and *Lachnoclostridium* 10 in the HN group were significantly lower than in the other two groups ($P < 0.05$). The relative abundance of *Succiniclasticum* in the LN group was significantly higher than in the other groups ($P < 0.05$). The MN group exhibited significantly higher relative abundances of *Lysinibacillus*, *Bacillus*, and *Phyllobacterium* compared to the other groups ($P < 0.05$), and the relative abundance of *Victivallis* was extremely significantly higher in the MN group than in the other groups ($P < 0.01$). These findings indicate that dietary NDF levels ranging from 35.01% to 45.16% significantly affect ruminal NH -N concentration, acetate-to-propionate ratio, and the relative abundance of various bacterial genera, including *Prevotellaceae* UCG-001 and *Prevotellaceae* UCG-003, in goats.

Keywords: neutral detergent fiber; bacteria; high-throughput sequencing; fermentation parameters

Introduction

Fiber constitutes a substantial proportion of ruminant diets and plays an irreplaceable role in promoting rumen motility and maintaining normal rumen pH. Although numerous studies have investigated the physiological functions of fiber, previous definitions of fiber were neither scientific nor standardized. Currently, neutral detergent fiber (NDF) is widely recognized as the best indicator of dietary fiber level in animal nutrition because it encompasses nearly all fiber components. NDF not only maintains gastrointestinal health but also provides substantial energy for animal growth and development through its degradation products. However, ruminants lack the intrinsic ability to digest NDF and rely entirely on rumen microorganisms for fiber utilization.

The structure and composition of rumen microbial communities determine the host' s capacity to digest and utilize NDF, while dietary NDF serves as a crucial substrate for microbial growth and reproduction. Previous studies on dietary fiber level effects on rumen microorganisms primarily employed traditional culture techniques and fingerprinting methods such as denaturing gradient gel electrophoresis. Culture techniques can only isolate microorganisms capable of growing in artificial media, yet approximately 99% of rumen microbes remain unculturable. Fingerprinting techniques are time-consuming, labor-intensive, and have low resolution, detecting only about 10% of dominant microorganisms. Both methods severely underestimate rumen microbial diversity, leaving the mechanisms by which dietary NDF level influences rumen microbial structure and composition poorly understood.

To address these limitations, this study employed the advanced Illumina HiSeq 250PE high-throughput sequencing technology to comprehensively reveal the effects of dietary NDF level on rumen bacterial structure and composition in

goats. The findings enhance our understanding of how rumen microorganisms adapt to changes in nutrient levels and how nutrient levels affect microbial diversity, providing a foundation for future strategies to promote rumen fiber degradation through microbial manipulation.

1.1 Experimental Animals and Management

Six healthy male Nubian goats (average age: 8 months; average body weight: 28.33 ± 3.77 kg) were used in this study. Diets were formulated according to the Chinese Feeding Standard of Meat-Producing Sheep and Goats (NY/T 816 –2004) to achieve a daily weight gain of 0.1 kg per animal. A 3 × 3 Latin square design was employed with three dietary treatments based on NDF level: low (LN group, 35.01%), medium (MN group, 40.10%), and high (HN group, 45.16%), with two goats per group. Dietary composition and nutrient levels are presented in . Dry matter (DM), crude protein (CP), calcium (Ca), and phosphorus (P) were analyzed according to AOAC methods, while NDF and acid detergent fiber (ADF) were determined using the method of Van Soest et al. Animals were housed individually and fed twice daily at 09:00 and 17:00, with free access to water.

1.2 Experimental Design and Sample Collection

The feeding trial consisted of three periods, each lasting 20 days (14-day pre-trial and 6-day formal trial). Following each formal trial period, rumen contents (approximately 50 mL) were collected at 2 hours after morning feeding using a 10-mm diameter plastic tube connected to a vacuum pump. A mouth opener was used to facilitate tube insertion into the rumen. Immediately after collection, pH was measured using a portable pH meter. Samples were then placed in nitrogen-filled bags on ice, vigorously shaken to ensure transfer of solid-associated microbes to the liquid phase, filtered through four layers of cheesecloth, snap-frozen in liquid nitrogen, and stored at -80 °C until analysis.

1.3 Rumen Fermentation Parameter Analysis

Ammonia nitrogen (NH -N) concentration was determined using the method of Broderick and Kang. Rumen fluid samples were pretreated, and a standard curve was prepared to establish a linear regression equation. After mixing, 80 L of centrifuged rumen fluid was added to test tubes, followed by 40 L methanol, 2.5 mL phenol, and 2.0 mL alkaline sodium hypochlorite solution. Samples were incubated at 37 °C for 10 minutes, held at room temperature for 10 minutes, and absorbance was measured at 650 nm using a microplate reader (SpectraMax M2, Molecular Devices, USA). NH -N concentration was calculated using the regression equation.

Volatile fatty acid (VFA) concentration was determined by gas chromatography (CP-3800, Varian, USA) according to the method of Li and Meng. After

pretreatment, 0.2 mL metaphosphoric acid and 40 L crotonic acid were added to standard solutions, mixed, held at 4 °C for 30 minutes, and centrifuged at 12,000 × g for 10 minutes. The supernatant (0.1 mL) was mixed with 0.9 mL methanol, filtered through a 0.22- m organic membrane, and analyzed for acetate, propionate, and butyrate content.

1.4 DNA Extraction and High-Throughput Sequencing

Total microbial DNA was extracted from 200 L of rumen fluid using a stool genomic DNA extraction kit (Tiangen Biotech, Beijing, China). The V4 region of the bacterial 16S rRNA gene was amplified using universal primers (515F: GTGCCAGCMGCCGCGGTAA; 806R: GGACTACHVGGGTWTCTAAT) in a 50- L reaction mixture containing: 1 L dNTP Mixture (10 mmol/L), 1.25 L each primer (10 mol/L), 1 L total DNA (50 ng/ L), 0.25 L Taq DNA Polymerase (5 U/ L, with Mg^2), 5 L 10× Taq Buffer, and ddH O to 50 L. PCR conditions were: initial denaturation at 95 °C for 2 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; final extension at 72 °C for 5 min; and cooling at 10 °C. Qualified PCR products were sent to Novogene Bioinformatics Technology (Beijing, China) for high-throughput sequencing on the Illumina HiSeq 250 PE platform.

1.5 Bioinformatics Analysis

Raw sequencing data were initially quality-filtered using QIIME 1.8.0 to remove low-quality sequences, barcodes, and primer sequences. Paired-end reads were merged using Mothur according to the method of Yáñez-Ruiz et al. Sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using the Uparse module in QIIME 1.8.0, with the most abundant sequence in each OTU selected as the representative sequence. Representative sequences were aligned against the RDP database (Release 11.1, http://rdp.cme.msu.edu/) to construct OTU tables. Taxonomic annotation was performed using the RDP Classifier, and bar charts of phylum-level composition were generated. Alpha diversity indices (Chao1, Shannon, and observed species) were calculated based on OTU tables, rep_set.tree files, and maximum sampling depth after removing chimeras and singletons. Rarefaction curves were plotted for each sample, and shared genus analysis was performed using R software to generate clustering heatmaps based on shared genus composition and proportions.

1.6 Statistical Analysis

Data were analyzed using the following general linear model: Y = + + + + (i = j = k = 1, 2, 3), where Y is the observed value, is the overall mean, , , and represent experimental period, goat group, and dietary NDF level, respectively, and is random error. Differences among groups were tested using ANOVA in SPSS 21.0, with dietary NDF level as the main factor and no interaction effects. Duncan' s multiple range test was used for post-hoc

comparisons. Results are expressed as means ± standard deviation. Significance was declared at $P < 0.05$, and extreme significance at $P < 0.01$.

2.1 Effects of Dietary NDF Level on Rumen Fermentation Parameters

As shown in , rumen pH did not differ significantly among groups ($P > 0.05$) but tended to increase with dietary NDF level. NH -N concentration in the HN group was extremely significantly lower than in the LN and MN groups ($P < 0.01$), with no significant difference between the latter two groups ($P > 0.05$). No significant differences were observed among groups for acetate, propionate, butyrate, or total VFA concentrations ($P > 0.05$). However, the acetate-to-propionate ratio tended to increase with dietary NDF level, reaching significance between the LN and HN groups ($P < 0.05$).

2.2.1 Sequencing Depth and OTU Analysis

A total of 1,125,746 high-quality sequences were obtained, with an average of 62,541 ± 9,024 sequences per sample. Clustering yielded 17,198 OTUs. The three groups shared 1,012 OTUs, while pairwise comparisons revealed 1,197 shared OTUs between LN and MN groups, and 1,083 shared OTUs between LN and HN groups and between MN and HN groups ([Figure 1: see original paper]).

2.2.2 Rarefaction Curves and Alpha Diversity Analysis

Rarefaction curves are presented in [Figure 2: see original paper]. At the sequencing depth of 30,154 reads, all curves plateaued, indicating that the sequencing depth adequately covered the microbial diversity in each sample. Alpha diversity indices calculated at this depth are shown in . No significant differences were observed among groups for Chao1 or Shannon indices ($P > 0.05$). However, the observed species index in the LN group was significantly higher than in the other two groups ($P < 0.05$), with no significant difference between the MN and HN groups ($P > 0.05$).

2.2.3 Rumen Bacterial Structure and Composition

Taxonomic annotation identified 23 phyla, 44 classes, 71 orders, 121 families, and 225 genera. At the phylum level, no significant differences in relative abundance were detected among groups ($P > 0.05$). Bacteroidetes, Firmicutes, and Proteobacteria were the dominant phyla across all groups, followed by Lentisphaerae and Tenericutes ([Figure 3: see original paper]).

At the genus level, *Prevotella* 1 and Rikenellaceae RC9 gut group were the two most abundant genera in all groups. After combining 215 genera with

relative abundance below 1% into an "others" category, genus-level composition is shown in [Figure 4: see original paper]. lists genera with significantly different relative abundances among groups. The relative abundances of *Prevotellaceae* UCG-001, *Prevotellaceae* UCG-003, and *Ruminococcaceae* UCG-014 increased with dietary NDF level and were significantly higher in the HN group than in the other two groups ($P < 0.05$). *Ruminococcaceae* NK4A214 group and *Ruminococcaceae* UCG-005 also increased with NDF level and were significantly higher in the HN group than in the LN group ($P < 0.05$). Conversely, SP3-e08 and *Lachnoclostridium* 10 decreased with NDF level and were significantly lower in the HN group than in the other groups ($P < 0.05$). The relative abundance of *Succiniclasticum* was significantly higher in the LN group than in the other groups ($P < 0.05$). The MN group showed significantly higher relative abundances of *Lysinibacillus*, *Bacillus*, and *Phyllobacterium* compared to the other groups ($P < 0.05$), and the relative abundance of *Victivallis* was extremely significantly higher in the MN group than in the other groups ($P < 0.01$). The relative abundance of *[Eubacterium] ruminantium* group was significantly higher in the LN group than in the HN group ($P < 0.05$) but did not differ significantly from the MN group ($P > 0.05$).

2.2.4 Shared Genus Analysis

A total of 35 genera were shared across all samples. The major shared genera (relative abundance > 1%) were, in descending order: *Prevotella* 1 [(37.29 ± 7.27)%], Rikenellaceae RC9 gut group [(7.30 ± 0.54)%], *Prevotellaceae* UCG-003 [(2.31 ± 0.88)%], Lachnospiraceae ND3007 group [(2.19 ± 0.52)%], *Prevotellaceae* UCG-001 [(2.04 ± 0.93)%], Succinivibrionaceae UCG-002 [(1.90 ± 1.75)%], *Selenomonas* 1 [(1.79 ± 4.24)%], *Ruminococcus* 2 [(1.35 ± 1.77)%], *Succinivibrio* [(1.20 ± 0.58)%], and *Ruminococcaceae* UCG-014 [(1.13 ± 0.81)%]. These ten genera accounted for 56.50% of the total bacterial community. A clustering heatmap of shared genera is shown in [Figure 5: see original paper].

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3 Discussion

Dietary NDF regulates and maintains normal rumen fermentation; consequently, different NDF levels affect fermentation patterns. Narenbatu et al. used a completely randomized design to study rumen fermentation in Inner Mongolian cashmere goats fed six dietary NDF levels (49%, 52%, 55%, 59%, 62%, and 65%). They found that dietary NDF level significantly affected rumen pH and NH -N concentration but not microbial crude protein (MCP) or VFA concentrations. Similarly, Wang et al. investigated the effects of three dietary NDF levels (42.71%, 54.59%, 64.38%) on the rumen environment of Sunit sheep and observed that increasing NDF level significantly decreased NH -N concentration and increased the acetate-to-propionate ratio, consistent with our findings.

This study revealed that the relative abundances of *Ruminococcaceae* NK4A214 group, *Ruminococcaceae* UCG-005, and *Ruminococcaceae* UCG-014 increased with dietary NDF level. These bacteria belong to the family *Ruminococcaceae*, which has been shown to be closely associated with fiber degradation, producing cellulases that break down cellobiose and other fibrous materials. Patra et al. demonstrated that reduced *Ruminococcaceae* abundance decreases fiber digestibility, while Zhao et al. confirmed a significant correlation between *Ruminococcaceae* and dietary NDF digestibility. Our results suggest that increased dietary NDF level enhances *Ruminococcaceae* proliferation, likely through substrate induction effects, corroborating the close relationship between this family and rumen fiber degradation.

The relative abundances of *Prevotellaceae* UCG-001 and *Prevotellaceae* UCG-003 also increased with dietary NDF level. These bacteria belong to the genus *Prevotella*. Previous in vitro studies have shown that *Prevotella* species possess protein and amino acid-digesting activity and can indirectly promote fiber degradation when co-cultured with fiber-degrading bacteria, despite lacking direct fiber-degrading capacity. Zhao et al. reported that dietary fiber digestibility affects the abundance of these bacteria in calves. Combined with our results, this suggests that these bacteria may be important collaborators in fiber degradation, obtaining nutrients from the fiber degradation process and thus proliferating more in the HN group.

We also observed significant differences in *Succiniclasticum* abundance among groups. *Succiniclasticum* can ferment fiber or cellobiose to produce succinate, acetate, and CO , making it a typical fiber-degrading bacterium. Previous studies have demonstrated its close association with fiber degradation, and our earlier work showed that goats with higher fiber digestibility had greater *Succiniclasticum* abundance. Theoretically, increased dietary fiber should promote its growth. However, the LN group exhibited significantly higher abundance than the other groups, possibly due to the higher starch content in the LN diet. Starch is a primary substrate for *Succiniclasticum* and may stimulate its growth more effectively than fiber.

Conclusions

1. When dietary NDF level varied from 35.01% to 45.16%, increasing NDF level decreased rumen NH -N concentration and increased the acetate-to-propionate ratio in goats.
2. The dominant rumen bacterial phyla were Bacteroidetes, Firmicutes, and Proteobacteria.
3. Dietary NDF level significantly affected the relative abundance of 13 bacterial genera in goat rumen, with *Prevotellaceae* UCG-001, *Prevotellaceae* UCG-003, and *Ruminococcaceae* UCG-014 showing increased abundance

with higher NDF levels.

References

[1] ANNISON G. The role of wheat non-starch polysaccharides in broiler nutrition[J]. Australian Journal of Agricultural Research, 1993, 44(3): 405-422.

[2] 周祥. 日粮不同 NDF 水平对杂交水牛瘤胃细菌群落结构与瘤胃主要功能细菌的影响 [D]. 硕士学业论文. 武汉: 华中农业大学, 2015.

[3] JAMI E, MIZRAHI I. Composition and similarity of bovine rumen microbiota across individual animals[J]. PLoS One, 2012, 7(3): e33306.

[4] PITTA D W, KUMAR S, VEICCHIARELLI B, et al. Bacterial diversity associated with feeding dry forage at different dietary concentrations in the rumen contents of Mehshana buffalo (Bubalus bubalis) using 16S pyrotags[J]. Anaerobe, 2014, 25: 31-41.

[5] 曹晨霞, 韩琬, 张和平. 第三代测序技术在微生物研究中的应用 [J]. 微生物学通报, 2016, 43(10): 2269-2276.

[6] YANG L Y, CHEN J, CHENG X L, et al. Phylogenetic analysis of 16S rRNA gene sequences reveals rumen bacterial diversity in Yaks (Bos grunniens)[J]. Molecular Biology Reports, 2010, 37(1): 553-562.

[7] AOAC. Official methods of analysis[M]. 15th ed. AOAC International, 1990.

[8] VAN SOEST P J, ROBERTSON J B, LEWIS B A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition[J]. Journal of Dairy Science, 1991, 74(10): 3583-3597.

[9] 王继文, 王立志, 闫天海, 等. 山羊瘤胃与粪便微生物多样性 [J]. 动物营养学报, 2015, 27(8): 2559-2571.

[10] WANG L Z, WANG Z S, XUE B, et al. Comparison of rumen archaeal diversity in adult and elderly yaks (Bos grunniens) using 16S rRNA gene high-throughput sequencing[J]. Journal of Integrative Agriculture, 2017, 16(5): 1130-1137.

[11] BRODERICK G A, KANG J H. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media[J]. Journal of Dairy Science, 1980, 63(1): 64-75.

[12] LI Y L, MENG Q X. Effect of different types of fibre supplemented with sunflower oil on ruminal fermentation and production of conjugated linoleic acids in vitro[J]. Archives of Animal Nutrition, 2006, 60(5): 402-411.

[13] CAPORASO J G, LAUBER C L, WALTERS W A, et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample[J]. Pro-

ceedings of the National Academy of Sciences of the United States of America, 2011, 108(1S): 4516-4522.

[14] WRIGHT A D G, WILLIAMS A J, WINDER B, et al. Molecular diversity of rumen Methanogens from sheep in western Australia[J]. Applied and Environmental Microbiology, 2004, 70(3): 1263-1270.

[15] YÁÑEZ-RUIZ D R, MACÍAS B, PINLOCHE E, et al. The persistence of bacterial and Methanogenic archaeal communities residing in the rumen of young lambs[J]. FEMS Microbiology Ecology, 2010, 72(2): 272-278.

[16] 那仁巴图, 贾志海, 卢德勋, 等. 不同纤维水平口粮对内蒙古白绒山羊瘤胃发酵的影响 [J]. 中国畜牧杂志, 2008, 44(3): 27-30.

[17] 王海荣, 侯先志, 王贞贞, 等. 不同纤维水平日粮对绵羊瘤胃内环境的影响 [J]. 内蒙古农业大学学报: 自然科学版, 2008, 29(3): 9-14.

[18] WOOD T M. Cellulase of Ruminococcus albus[J]. Methods in Enzymology, 1988, 160: 216-221.

[19] EZAKI T. Ruminococcus[M]. New York: John Wiley & Sons, Ltd, 2015.

[20] PATRA A K, YU Z T. Essential oils affect populations of some rumen bacteria in vitro as revealed by microarray (rumen bact array) analysis[J]. Frontiers in Microbiology, 2015, 6: 297.

[21] ZHAO X H, CHEN Z D, ZHOU S, et al. Effects of daidzein on performance, serum metabolites, nutrient digestibility, and fecal bacterial community in bull calves[J]. Animal Feed Science and Technology, 2017, 225: 87-96.

[22] SCHWINGEL W R, BATES D B. Use of sodium dodecyl sulfate polyacrylamide gel electrophoresis to measure degradation of soluble soybean proteins by Prevotella ruminicola GA33 or mixed ruminal microbes in vitro[J]. Journal of Animal Science, 1996, 74(2): 475-482.

[23] BEKELE A Z, KOIKE S, KOBAYASHI Y. Genetic diversity and diet specificity of ruminal Prevotella revealed by 16S rRNA gene-based analysis[J]. FEMS Microbiology Letters, 2010, 305(1): 49-57.

[24] ROSENBERG E. The family Prevotellaceae[M]//ROSENBERG E, DELONG E F, LORY S, et al. The Prokaryotes. Berlin: Springer, 2014.

[25] RAINEY F A. Succiniclasticum[M]//ONYENWOKE R U, WIEGEL J. Bergey' s manual of systematics of archaea and bacteria. New York: John Wiley & Sons, Ltd., 2015.

[26] AN D D, DONG X Z, DONG Z Y. Prokaryote diversity in the rumen of yak (Bos grunniens) and Jinnan cattle (Bos taurus) estimated by 16s rDNA homology analyses[J]. Anaerobe, 2005, 11(4): 207-215.

[27] 刘利, 唐纪良, 冯家勋. 广西水牛瘤胃中的细菌多样性 [J]. 微生物学报, 2009, 49(2): 251-256.